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Inositol triphosphate produces different patterns of cytoplasmic Ca^{2+} spiking depending on its concentration

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In single mouse pancreatic acinar cells the effects of intracellular infusion of inositol 1,4,5-trisphosphate ($InsP_3$) or the non-metabolizable $InsP_3$ analogue inositol 1,4,5-triphosphorothioate ($InsPS_3$) have been investigated using a wide range of concentrations. Different types of cytosolic Ca^{2+} fluctuation patterns (monitored as Ca^{2+}-dependent Cl^- current in patch-clamp whole-cell recording experiments) could be generated by $InsP_3$ or $InsPS_3$, dependent on concentration, resembling those previously shown to be evoked by varying degrees of receptor activation in these cells. Low $InsPS_3$ concentrations evoked repetitive local Ca^{2+} spikes whereas at relatively high concentrations repetitive Ca^{2+} waves were produced. In the presence of intracellular citrate a much lower messenger level was sufficient to generate waves. The $InsP_3$ concentration determines whether the cytosolic Ca^{2+} signals are local or global.

Ca^{2+} spike; Ca^{2+} wave; Inositol trisphosphate; Inositol trisphosphorothioate

1. INTRODUCTION

Activation of receptors linked to the enzyme phospholipase C (PLC) evokes repetitive transient spikes in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) [1]. Two quantitative models seem capable of explaining Ca^{2+} spike generation. In the inositol 1,4,5-triphosphate ($InsP_3$)- Ca^{2+} crosscoupling (ICC) model the receptor-triggered formation of $InsP_3$ leads to a rise in $[Ca^{2+}]_i$ that accelerates $InsP_3$ formation via Ca^{2+}-activation of PLC, creating a positive feedback loop [2,3]. In the Ca^{2+}-induced Ca^{2+}-release (CICR) model the primary $InsP_3$-evoked rise in $[Ca^{2+}]_i$ opens channels in an $InsP_3$-insensitive store. Here positive feedback comes from Ca^{2+} catalyzing its own release [1,4,5].

The ICC model requires that the $InsP_3$ concentration like $[Ca^{2+}]_i$ undergoes spiking [2], but experimental evidence from pancreatic acinar cells shows that intracellular infusion of the non-metabolizable $InsP_3$ analogue inositol 1,4,5-trisphosphorothioate ($InsPS_3$) evokes regular Ca^{2+} spikes [6]. This appears to rule out the ICC model for these cells. Nevertheless Meyer and Stryer [2] have stressed that in the pancreatic experiments [6] the $InsPS_3$-evoked spikes were of a much shorter duration than those often evoked by receptor activation. In the pancreatic acinar cells low concentrations of acetylcholine (ACh) as well as $InsP_3$ or $InsPS_3$ evoke local short-lasting Ca^{2+} spikes near the cell membrane whereas higher ACh concentrations or cholecystokinin (CCK) can evoke longer transient Ca^{2+} signals that spread as waves throughout a cell or even a coupled acinar network. Each Ca^{2+} wave is triggered by a short-lasting local spike [7–10]. It is not known whether a constant level of $InsP_3$ can generate such repetitive global Ca^{2+} transients. We now show that intracellularly applied $InsP_3$ or $InsPS_3$ can evoke a number of different cytoplasmic Ca^{2+} signal patterns depending on the concentration of the messenger. A relatively high $InsPS_3$ concentration is required in order to evoke repetitive long Ca^{2+} transients, but in the presence of intracellular citrate a much lower $InsPS_3$ level is sufficient.

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2. MATERIALS AND METHODS

Fragments of mouse pancreas were digested by pure collagenase, washed and pipetted to produce single acinar cells as previously described [4,6,7]. The tight-seal, whole-cell current configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells as previously described in detail for pancreatic acinar cell studies [11]. Patch-clamp pipettes had resistances of 2–4 MΩ and the access resistance to the cell interior was about 5–10 MΩ [11]. Ca^{2+}-dependent Cl^- currents were measured with the two-voltage pulse protocol as described previously [9]. The extracellular solution contained (mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.0, $MgCl_2$, HEPES 10 (pH 7.2) and glucose 10. The intracellular pipette solution contained (mM): KCl 140, Na_2ATP 1, $MgCl_2$ 1.13, glucose 10 and HEPES 10 (pH 7.2). EGTA (10 μM) was also present.

In experiments where 10 mM citrate was present the KCl concentration was reduced so as to keep the osmolarity at the control level. D-Ins 1,4,5-PS_3 was synthesized by a method identical to that previously described for the racemate [12] except that optically pure L-1,2,4-tri-*O*-benzyl-*myo*-inositol [13] was employed for phosphorylation. After deblocking of protecting groups, crude D-Ins 1,4,5-PS_3 was purified on a column of DEAE Sephadex A-25 resin using a gradient of triethylammonium bicarbonate, pH 8. After elution the pure phosphorothioate was quantified by quantitative phosphate analysis and stored as the triethylammonium salt.

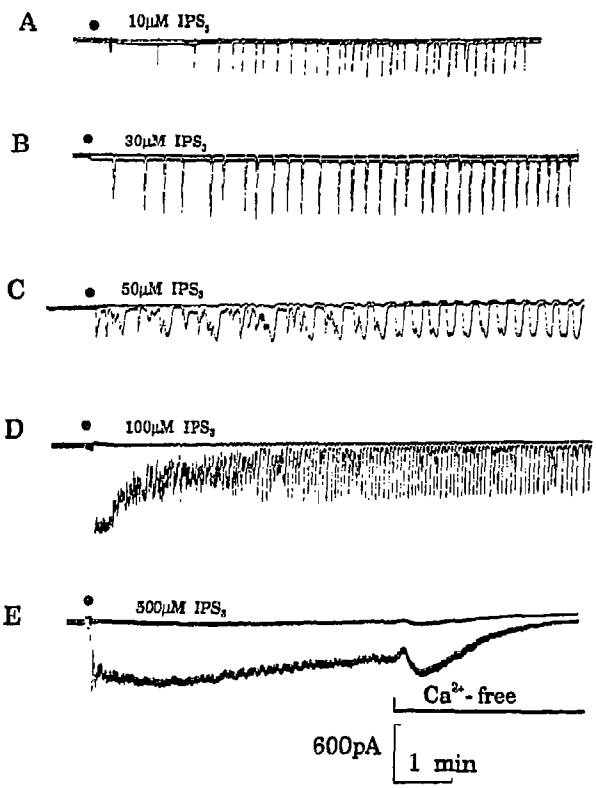

Fig. 1. Different patterns of cytoplasmic Ca^{2+} fluctuations monitored as Ca^{2+}-dependent Cl^- current evoked by internal application of $InsPS_3$ (IPS_3) in single mouse pancreatic acinar cells. The cells were voltage-clamped at a holding potential of −30 mV and depolarizing voltage jumps of 150 ms duration to a membrane potential of 0 mV were repetitively applied throughout all experiments. Because of the compression of the current traces the records seem to show currents at −30 mV (bottom traces) and 0 mV (top traces) simultaneously. At 0 mV there are only very small current fluctuations as the Cl^- equilibrium potential (E_{Cl^-}) is close to zero. At −30 mV there is a large electrical gradient favouring Cl^- efflux and when the Ca^{2+}-dependent Cl^- channels open, due to increases in $[Ca^{2+}]_i$, inward currents (downward deflections) are seen. The panels A–E represent individual experiments in which the internal (pipette) solution contained 10, 30, 50, 100, or 500 μM IPS_3, respectively. The filled circle at the beginning of each panel indicates the time of establishment of the whole-cell recording configuation (start of IPS_3 diffusion into cell interior). In (E) the bar labelled Ca^{2+}-free indicates that the external solution did not contain Ca^{2+} and that EGTA (1 mM) was present.

3. RESULTS

Fig. 1 shows examples of the various patterns evoked by $InsPS_3$. The traces shown for 10, 30 and 500 μM $InsPS_3$ are typical, but in the experiments with 50 and 100 μM $InsPS_3$ a variety of patterns ranging from repetitive short-lasting spikes to sustained responses were obtained. Table I summarizes all the results. The dose–response curve in the concentration range 30–100 μM is very steep, but the important point is that 50 or 100 μM $InsPS_3$ can evoke a pattern of repetitive long transients whereas at 10 or 30 μM repetitive short-lasting spikes are invariably produced. We used $InsPS_3$ rather than $InsP_3$ since the natural messenger is rapidly metabolized [1,14]. The synthetic analogue in which the phosphate groups have been replaced by phosphorothioate groups [12] confers resistance to phosphatase and kinase-mediated metabolism [14]. $InsPS_3$ is a full agonist at releasing intracellular Ca^{2+} and is only 3- to 6-fold less potent than the natural messenger [14]. $InsPS_3$ displaces $[^3H]InsP_3$ from cerebellar membranes with an IC_{50} value that is about 5 times higher than $InsP_3$ [15].

Although $InsP_3$ is most likely metabolized when infused into cells we carried out a few experiments to see if different concentrations of the natural messenger could also evoke different cytoplasmic Ca^{2+} fluctuation patterns. In the two experiments with 10 μM $InsP_3$ in the pipette solution repetitive short-lasting spikes were seen (Fig. 2A) confirming the results of the 12 experiments previously reported by us [4]. In the 4 experiments with 50 or 100 μM $InsP_3$ there was initially a quasi-sustained response followed by a somewhat irre-

Table I

Cytoplasmic Ca^{2+} fluctuation patterns evoked by stimulation with D-Ins 1,4,5-PS_3 ($InsPS_3$)

Stimulus	Number of cells investigated	Response	Number of cells showing response
10 μM $InsPS_3$	11	Short-lasting spikes	11
10 μM $InsPS_3$ in the presence of 10 mM citrate	4	Long transients + spikes	4
30 μM $InsPS_3$	9	Short-lasting spikes	9
50 μM $InsPS_3$	17	Short-lasting spikes	5
		Long transients + spikes	3
		Sustained	9
100 μM $InsPS_3$	13	Short-lasting spikes	3
		Long transients + spikes	5
		Sustained	5
500 μM $InsPS_3$	7	Sustained	7

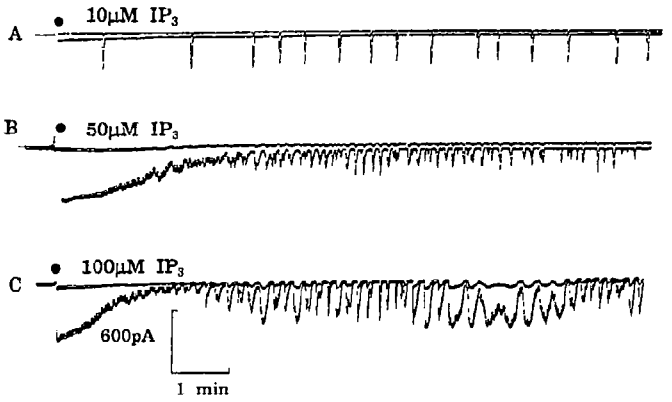

Fig. 2. Different Ca^{2+} fluctuation patterns evoked by different concentrations of $InsP_3$ (IP_3). Panels A–C represent individual experiments in which the internal (pipette) solution contained 10, 50 and 100 μM IP_3, respectively.

gular mixed pattern of short spikes and longer transients (Fig. 2B and C).

The repetitive short-lasting spikes evoked by low concentrations of $InsPS_3$ or $InsP_3$ are insensitive to removal of extracellular Ca^{2+} during the initial 10-min period of stimulation [6] although later an extracellular Ca^{2+} dependency develops [16]. Removal of external Ca^{2+} results, after a brief transient increase, in a gradual and severe reduction of the sustained responses (Fig. 1E). When a high concentration (1 mM) of the Ca^{2+} chelator EGTA was present in the external Ca^{2+}-free solution (as in Fig. 1E) the response could not be brought back after Ca^{2+} re-admission, but when the EGTA concentration was reduced to 0.2 mM the effect was reversible.

The probability of ACh-evoked short-lasting Ca^{2+} spikes triggering longer transients is dramatically increased by intracellular perfusion with solutions containing a high concentration of the mobile low-affinity Ca^{2+} buffer citrate [9]. Since the $InsPS_3$ concentration of 50 μM (corresponding to 10 μM $InsP_3$) required to produce long transients (Fig. 1C) is relatively high (in permeabilized pancreatic acinar cells 5 μM $InsP_3$ appears to be a maximal dose [17]) we investigated the effect of a low $InsPS_3$ concentration in the presence of citrate. Fig. 3 compares the effects of $InsPS_3$ in control and citrate pipette solutions and shows that with 10 mM citrate in the perfusion solution 10 μM $InsPS_3$ (corresponding to an $InsP_3$ concentration of 2 μM) evokes a pattern of free-standing slim spikes mixed with spikes followed by much longer transients (Table I). In the absence of citrate such an effect can be induced by addition of CCK (Fig. 3A) [9]. Irrespective of the mechanism by which citrate acts these results show that the precise $InsP_3$ concentration required to produce a particular cytoplasmic Ca^{2+} fluctuation pattern may depend on soluble cytosolic components that could be subject to wash-out during whole-cell recording.

4. DISCUSSION

Our results show that a constant concentration of the Ca^{2+}-releasing messenger $InsP_3$ can generate a number of different cytosolic Ca^{2+} spike and wave patterns, depending on its concentration, resembling those previously shown to be evoked by varying degrees of receptor activation [7,9,10]. The spreading of the local short-lasting Ca^{2+} spikes can be prevented by non-mobile cytosolic buffers, uptake into intracellular organelles and/or extrusion through the cell membrane [2,9,18]. Mobile Ca^{2+} buffers can facilitate Ca^{2+} diffusion by picking up Ca^{2+} at the high end of a steady gradient and then diffuse with its bound Ca^{2+} to the low end where Ca^{2+} will be released [9] and this process may explain the result shown in Fig. 3, but how can an increase in $InsP_3$ concentration promote spreading and thereby account for the generation of the longer Ca^{2+} transients (Figs. 1 and 2)?

The simplest explanation is provided by the quantal nature of $InsP_3$-evoked intracellular Ca^{2+} release [17] in conjunction with the recent finding that an elevation of cytoplasmic Ca^{2+} concentration can evoke further Ca^{2+} release not only through $InsP_3$-insensitive caffeine-sensitive channels [4,5,19] but also via $InsP_3$-sensitive pores [19] in the endoplasmic reticulum. In pancreatic cells a small steady Ca^{2+} release evoked by a low $InsP_3$ concentration generates local repetitive Ca^{2+}-induced Ca^{2+} spikes according to the two-pool model [1,4,16,20] and mainly via $InsP_3$-insensitive channels since intracellular Ca^{2+} infusion can mimic this effect and the Ca^{2+}-evoked Ca^{2+} spikes cannot be blocked by infusion of the $InsP_3$ antagonist heparin [4]. A higher $InsP_3$ concentration would release Ca^{2+} from pools not previously accessible [17] and effectively allow further Ca^{2+}-induced Ca^{2+} release also via $InsP_3$-sensitive channels [19] thereby explaining the spreading of the Ca^{2+} signal.

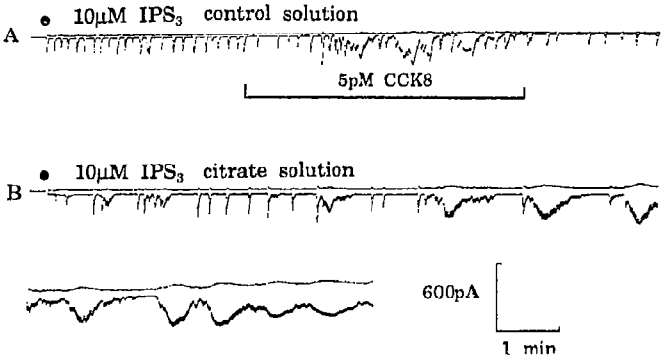

Fig. 3. Comparison of the effects of 10 μM $InsPS_3$ (IPS_3) in a control pipette solution (A) and a pipette solution containing 10 mM citrate (B). In the control experiment (A) IPS_3 (10 μM) evokes repetitive slim spikes, but addition of 5 pM CCK8 externally evoked longer transients whereas in the citrate experiment (B) the same concentration of IPS_3 by itself is able to induce longer Ca^{2+} transients. The bottom part of panel B is a direct continuation of the upper part.

Certain types of receptor activation may in addition to generating $InsP_3$ also control Ca^{2+} spreading by regulation of Ca^{2+} extrusion and/or Ca^{2+} re-uptake into organelles or by the production of a caffeine-like substance (spreading factor) promoting Ca^{2+}-induced Ca^{2+} release [9]. Our results indicate that $InsP_3$ is itself a spreading factor, but additional receptor-mediated controls of Ca^{2+} transport are most likely required to fully account for the different shapes of Ca^{2+} transients evoked by different agonists [9,21,22].

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